ELECTRON MICROSCOPE STUDY OF THE DISTAL PORTION OF A PLANARIAN RETINULAR CELL[1]

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With the increasing development of techniques of electron microscopy, ever more attention has been devoted to the study of the fine structure of photoreceptors, the details of which are beyond the limit of resolution of the light microscope. One such structure is the visual receptor of the eye of the planarian, *Dugesia tigrina*.

The classical study which has served as a model for many textbook illustrations of the planarian eye is the work of Hesse (1897), whose description of the visual end organ was subsequently modified by Taliaferro (1920). The latter investigator also posed the possibility of analogizing, and possibly homologizing, the retinulae of the turbellarian eye with the receptors of the vertebrate eye. Analogies between vertebrate and invertebrate eyes have been extended by Wolken (1958), who deemed the fine structure of the sensory cells of the planarian eye to resemble the outermost portions of vertebrate retinal components. Since preliminary studies by the present author tended to contradict this view, an electron microscopic examination of the planarian eye was deemed useful not only from the point of view of clarifying its morphology, but also from the standpoint of providing evidence as to a possible analogy of platyhelminth and chordate eyes.

Materials and Methods

Specimens of *Dugesia tigrina* were cut in two transversely at a level just behind the auricles, and the anterior portions were fixed immediately in a solution of 1% osmium tetroxide buffered at a pH of approximately 7.2 with a veronal acetate buffer. Following a period of fixation ranging from 20 minutes to 2 hours, the specimens were washed in distilled water, dehydrated in ethanol, and embedded in a mixture of 30% methyl methacrylate and 70% N-butyl methacrylate.

Sections, cut on an International Minot rotary microtome set to cut at 0.025 μ, were mounted on grids previously coated with a thin collodion membrane. The electron microscope used was an RCA model EMU 2.

Material for study with the light microscope was fixed in Bouin's fixative, dehydrated in ethanol, and embedded in paraffin. Sections were cut at 6 μ and stained with Heidenhain's iron hematoxylin.

Description

Observations with the light microscope show that the portion of the retinula (R, Fig. 1) found within the pigment cup exhibits a different capacity for staining

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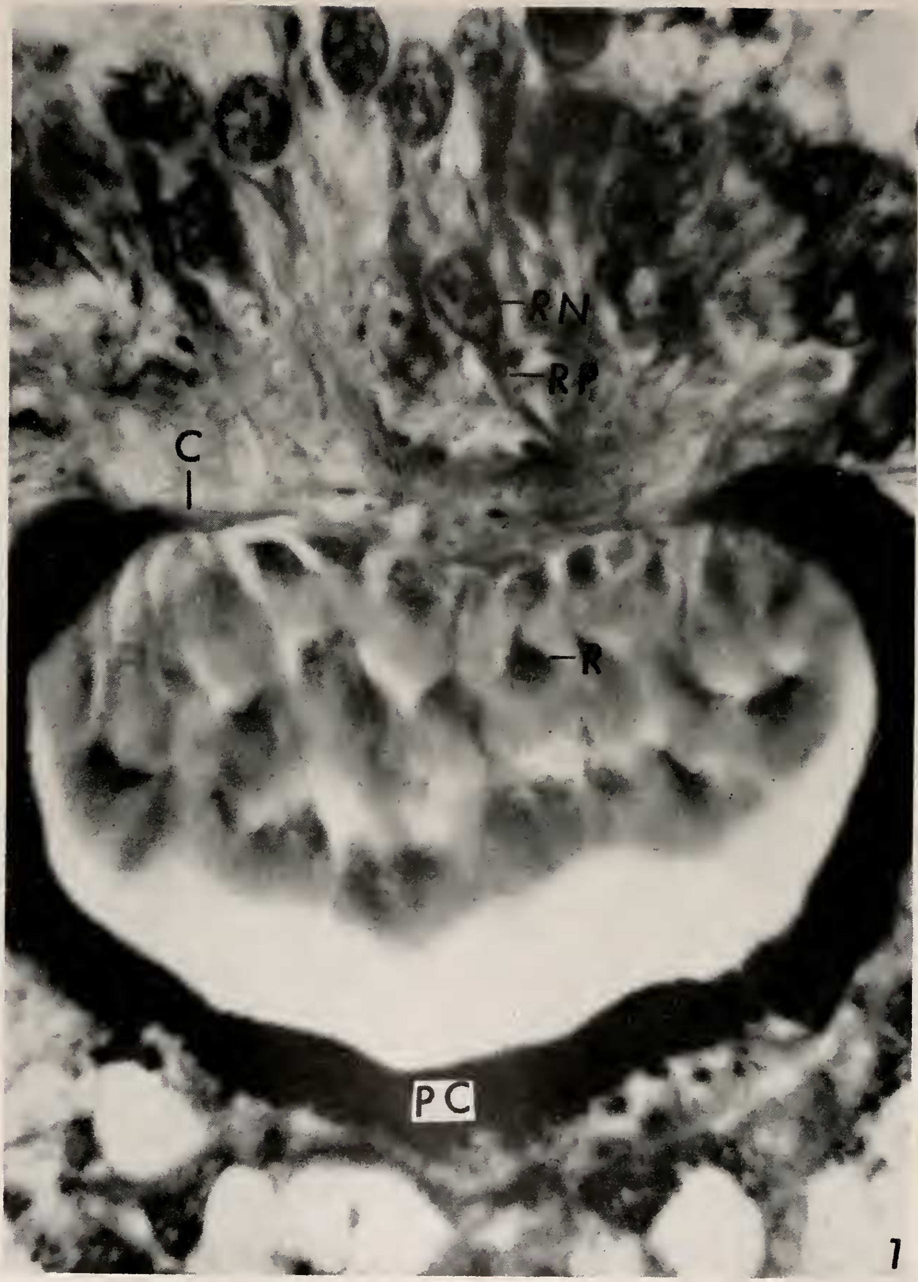

FIGURE 1. Light micrograph of an approximately frontal section, showing the pigment cup (PC), covering of the aperture of the pigment cup (C), retinula (R), nucleus of retinula (RN), and process of retinula (RP). The space between the most distal portions of the retinulae and the inner surface of the pigment cup is probably an artifact induced by fixation. × 1870.

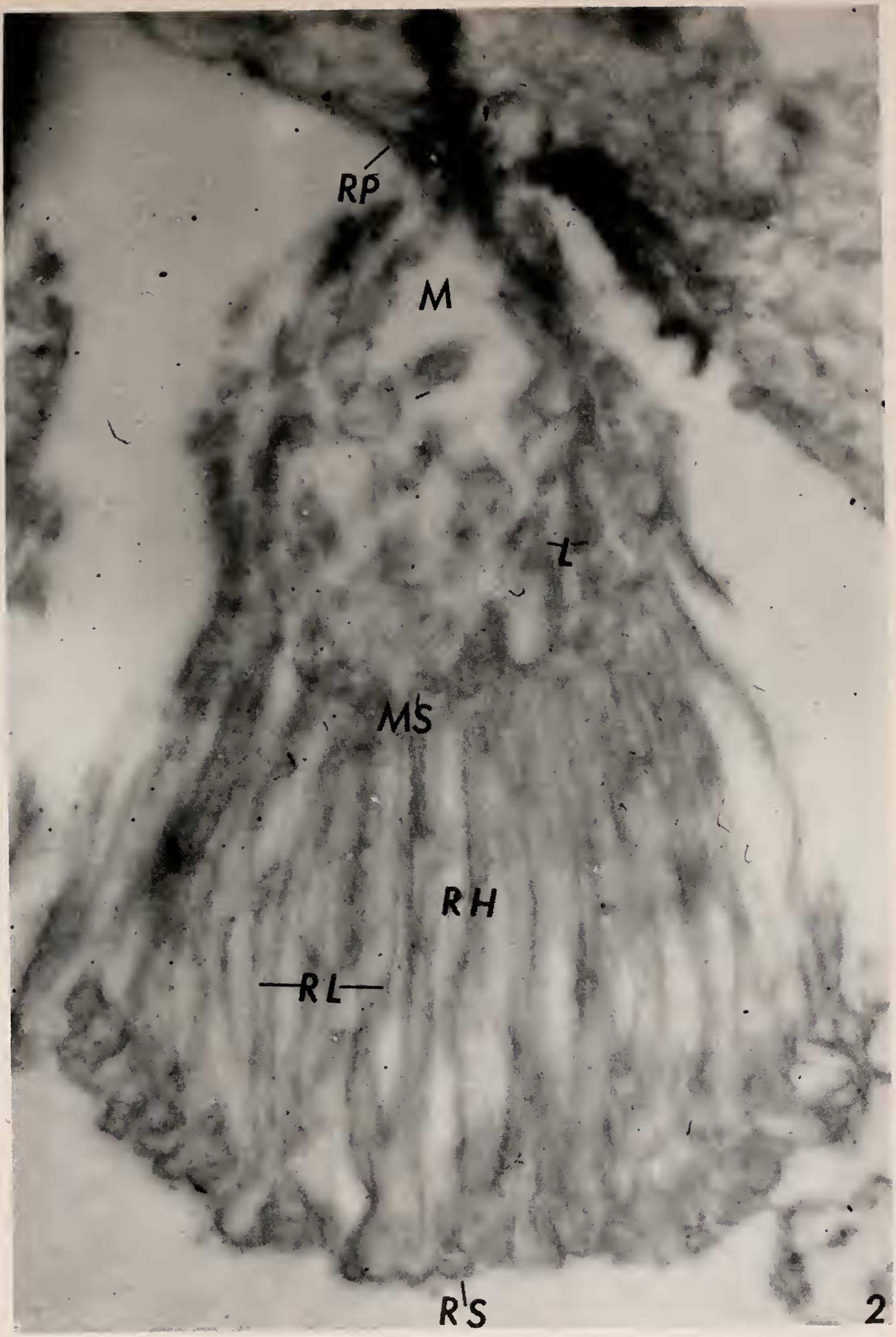

FIGURE 2. Electron micrograph of an approximately longitudinal section through a retinula, showing the retinular process (RP), the lamellae (L) of the "middle region" (M), and the bulb-like swellings (RS) at the distal portions of the lamellae (RL) of the rhabdome (RH). A delicate membrane (MS) separates the middle and rhabdome regions of the retinula. × 20,900.

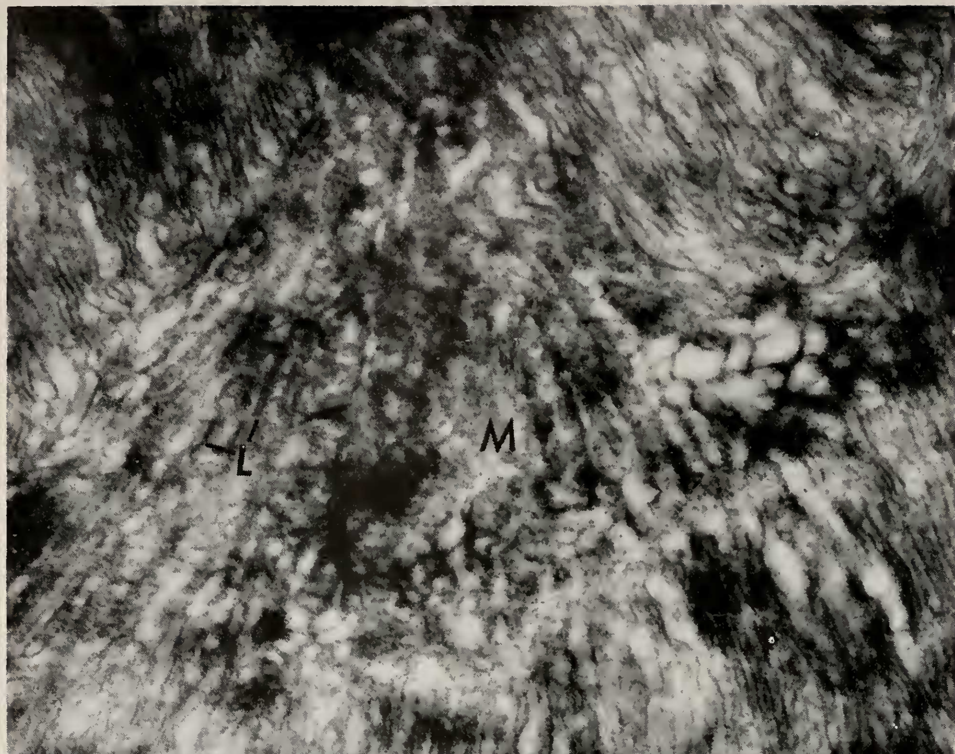

FIGURE 3. Electron micrograph of an approximately longitudinal section through a retinula, showing the lamellae (L) of the "middle region" (M), and the lamellae (RL) of the rhabdome (RH). Portions of rhabdomes of other retinulae are evident in the upper corners of the figure. × 19,000.

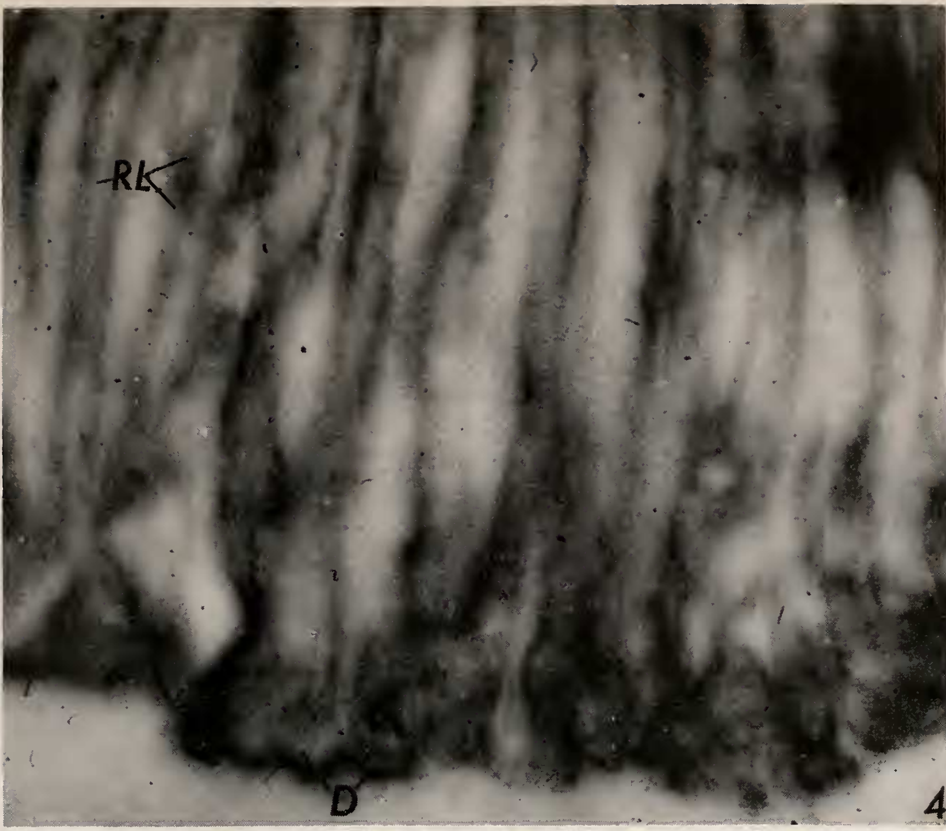

FIGURE 4. Distal portion of rhabdome of retinula seen in Figure 2, here shown at higher magnification, indicating the lamellae (D) in the distal swellings of rhabdome lamellae (RL). × 35,000.

FIGURE 5. Approximately transverse section through a portion of the "middle region" of a retinula, showing the lamellae (L) of that region. × 30,000.

with Heidenhain's iron hematoxylin from the rest of the retinula. The most distal portion of the sensory cell, which has been called the rhabdome by Taliaferro (1920), retains little of the stain and exhibits no internal structure save what has been termed a "rod border" (Hesse, 1897) or longitudinally oriented "striae" (Taliaferro, 1920). Proximal to the rhabdome of the retinula is a "middle region" staining intensely with iron hematoxylin, which continues proximally as a narrow process of the retinula. This latter process remains moderately intensely stained.

The electron microscope studies reveal that the whole of the rhabdome of the visual sensory cell consists of longitudinally oriented lamellae (RL, Figs. 2–4). These lamellae range in length from 3.18 μ to 4.20 μ, and in thickness from 300 Å to 490 Å. The distal portions of some lamellae are further differentiated into bulb-like swellings (RS, Fig. 2) bearing smaller lamellar structures with a maximum length of 0.44 μ that vary in thickness from 140 Å to 230 Å (D, Fig. 4).

A delicate membrane appears to delimit the "middle" from the rhabdome of the retinula (MS, Fig. 2). The sole discrete structures within the "middle region" (M, Figs. 2 and 3) are fiber-like lamellae (L, Figs. 2, 3, and 5). These lamellae vary in thickness from 160 Å to 610 Å, and appear in several instances to extend into the rhabdome.

Discussion

It has been suggested that the rhabdome of the retinula is its photosensitive region (Hesse, 1897; Taliaferro, 1920). In the visual biochemical reactions which presumably occur in the rhabdome, a lamellar arrangement would present a large surface area, particularly in a direction perpendicular to the longitudinal axes of the rhabdome vesicles. However, Taliaferro, in his experiments involving the locomotor responses of *Planaria* to light, has concluded (p. 113) that, "Light must strike a given rhabdome parallel with its longitudinal axis in order to cause stimulation of the rhabdome." But this direction is precisely the one which would least directly strike the longitudinal lamellar surfaces of the rhabdome. On the other hand, it is the most favorable direction by which light may reach the bulb-like swellings at the distal portions of the rhabdomal lamellae. The differentiated areas at the distal portions of the rhabdomal lamellae may be significant as possible sites of visual biochemical reaction.

The existence of a "middle region" as a distinct structure in the retinula of the planarian eye was not recognized by Hesse (1897), who figured in its place a fibrillar structure which was continuous distally with the "rod border" and proximally with fibers running to the cell body. In the preceding year Jänichen's (1896) description of the planarian eye showed a middle region in the retinula. In 1920, Taliaferro emphasized the importance of the "middle region," stating (p. 105), "that possibly this region serves as a crude lens to concentrate the rays of light upon the sensitive rhabdome and that photic stimulation depends upon this." Another possibility, however, is that these lamellae may transmit the impulse propagated by light-stimulation of the rhabdomal constituents. The latter explanation finds some support in the fact that some lamellae can be seen to continue into the rhabdome region.

As for the possibility of analogizing or homologizing the turbellarian and chordate eyes, the most that can be said is that certain similarities do exist between the two types. Both kinds of eye are of the inverse type, having the most distal portions of the receptors directed away from the opening of an eye partially lined with opaque pigment. Both kinds have visual receptors, each consisting of at least three distinct portions. The rods and cones of the vertebrate eye lie next to one another in approximately the same plane, forming a relatively flat retina lining part of the interior of the eye. In contrast, although the retinulae of the eye of *Dugesia tigrina* tend to be so oriented that their longitudinal axes are approximately normal to the nearest portion of the inner surface of the pigment cup, this orientation is far from consistent. In addition, the retinulae do not seem to lie in a single plane, but rather are distributed throughout the eye at varying distances from the inner surface of the pigment cup. Further, the fine structure of the planarian retinula differs markedly from that of the vertebrate rod or cone as described by several investigators (De Robertis and Lasansky, 1958; Sjöstrand, 1953; Wolken, 1958). Especially significant is the difference in orientation of the lamellar component, which is transverse in vertebrate receptors, but longitudinal in the planarian retinulae.

Summary

1. Anterior portions of *Dugesia tigrina* were prepared for electron microscopic examination by fixing in osmium tetroxide buffered at pH 7.2, embedding in methacrylate polymer, and sectioning at 0.025 μ.

2. The rhabdome of the retinula is composed of longitudinally oriented lamellae whose distal portions are differentiated into bulb-like swellings bearing smaller lamellae. The "middle region" of the retinula contains lamellae that appear, in some instances, to extend into the rhabdome.

3. Little analogy can be drawn between the fine structure of the retinula of the planarian eye and that of the rods and cones of the vertebrate eye.

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